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GENOTOXICITY ASSESSMENT OF NANO-PARTICLES ON MICROPROPAGATED OLIVE (*OLEA EUROPAEA* L.) PLANTS USING RAPD AND DAMD MARKERS

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Abstract

Nanomaterials have been widely used to improve the plant's growth *in vitro*. The aim of the current study was to evaluate positive and negative effects of nanotechnology in plant tissue culture by treatment of three olive cultivars 'Koroneiki', 'Picual' and 'Manzanillo' with different concentrations of silver or selenium nanoparticles(NPs), compared with the control. In addition, genotoxicity evaluation of nanoparticles on olive plants by molecular markers. Effect of AgNPs or SeNPs on genomic DNA was studied by Random amplified polymorphic DNA (RAPD) and Direct amplification of mini satellite-region DNA(DAMD) profiles, which allow detection of alterations in DNA sequence between treated and untreated olive plants. Differences in RAPD and DAMD assays were analyzed depending on the appearance or disappearance of bands. Amplification profiles of genomic DNA with molecular markers indicating that olive plantlets treated with (2.5 and 5 mg L^{-1}SeNPs) and (5 and 10 mg L^{-1}AgNPs) scored polymorphism which ranged from 41.10 to 41.46% using RAPD and DAMD techniques, respectively.This study is the first report stated that5 mg L^{-1}AgNPs are safe concentration for olive growth *in vitro*. In contrast, the high concentrations of AgNPs (10 mg L^{-1}) and SeNPs (2.5 and 5 mg L^{-1}) induced DNA change of olive plants which confirms the NPs as a potential environmental hazard.

Key words : Tissue cultures, silver nanoparticles, selenium nanoparticles, cluster analysis.

Introduction

The olive tree (*Olea europaea* L.) is one of the most important fruit crops in the Mediterranean basin (Fabbri *et al*., 2004), olive trees were asexual propagated by cuttings and grafting (Hartmann and Kester, 1975). Plant tissue cultureis a powerful technique which permits fast clonal propagation, production of pathogen-free plants, under controlled conditions and helps in the genetic improvement of crops (Vasil, 1988; Zuccherelli and Zuccherelli, 2002; Mangal *et al*., 2014; Abdallatif *et al*., 2015). Nanotechnology, a novel emerging field of science, allows advanced study and new applications in the field of agriculture and biotechnology (Sobha *et al*., 2010; Siddiqui *et al*., 2015). Nanoparticles (NPs) are produced in the size range of 1-100 nm (Roco, 2003). Nanomaterials have been broadly applied to enhance germination of seed, improve the growth of plants, yield and bioactive component content (Wang *et al*., 2016; Ruttkay-Nedecky *et al*., 2017). NPs have been confirmed to be advantageous for various contamination kinds elimination (Sondi and Salopek-Sondi, 2004; Jo *et al*., 2009; Wang *et al*., 2017; Spinoso-Castillo *et al*., 2017). Several reports have proven positive effects of NPs on thecallus induction, shoot multiplication and the plants growth promote *in vitro* (Al-Khayri and Al-Bahrany, 2001; Kumar *et al*., 2009; Aghdaei *et al*., 2012). AgNPs treatment increased survival and delayed explants senescence (Sarmast *et al*., 2015). Sharma *et al*., (2012) reported that incorporation of silver nanoparticles at 50 mg L^{-1} in MS

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basal medium enhanced the growth characteristics of mustard (*Brassica juncea*) plantlets by reduction the content of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and proline by activating antioxidant isozymes. The majority of nanomaterials cause toxicity at some concentrations by changing plant morpho-anatomical, physiological, biochemical and genetic constitutions (Puaand Chi, 1993; Rico *et al*., 2015). Several reports have indicated the phytotoxicity of NPs used at higher concentrations to plant tissues (Craig and Jason, 2011; Ghodake *et al*., 2011). NPs affect the mitotic activity and change the DNA profile andgene expression in the plants (Atha *et al*., 2012;Landa*et al*., 2015; Ewais *et al*., 2015; Tripathi *et al*., 2017). NPs phytotoxicity can be caused by the generation of reactive oxygen species (ROS), which results in lipid peroxidation, proteins and DNA damage (Arruda *et al*., 2015; Ma*et al*., 2015). Kokina*et al*., (2017) investigated the effect of AgNPs on somaclonal variation in the flax, somaclonal variation was higher in both callusand regenerated explants grown on medium containing NPs. López-Moreno *et al*., (2010) observed damage in the DNA structure in soybean plants in the presence of 2000 and 4000 mg/L CeO_2NPs. A similar effect was observed in the onion and tobacco (Ghosh *et al*., 2010) squash (Moreno-Olivas *et al*., 2014) and maize (Castiglione *et al*., 2011). Moreover, negative impacts were also shown in chromosome structures of maize (Castiglione *et al*., 2011). These kinds of damaging effects have been obtained by Random amplified polymorphic DNA (RAPD) technique, which uses to characterize and verify the origin, stability of clones and plants micropropagated by tissue culture technique (Pijut *et al*., 2007). The aim of the current study was to evaluate positive and negative effects of nanotechnology in olive plants micropropagated *in vitro* by treatment of three olive cultivars 'Koroneiki', 'Picual' and 'Manzanillo' with two different concentrations of silver or selenium nanoparticles, compared with the control. In addition, evaluation of genotoxicity for treating plants with NPs by RAPD and DAMD profiles.

Materials and Methods

Plant materials and explants preparation

The current research was carried out during (2018/19) seasons at the laboratory of Pomology Department, Faculty of Agriculture, Cairo University and Tissue culture technique Lab. Central Laboratories Network, Pomology Dept., and Genetic Engineering and Biotechnology Research Division, Genetics and Cytology Department, National Research Centre. Active spring shoots were collected from mature olive trees of cultivars namely 'Koroneiki', 'Picual' and 'Manzanillo', during the summer season. After removing of leaves; the shoots were cut into nodal segments. Surface sterilization performed according to Hassan *et al,* (2017).

In vitro propagation

Nodal segments of the selected olive cultivars were cultured on Rugini olive medium (Rugini, 1984) supplementedwith 2 mg L^{-1} of zeatin. Two types of nanoparticles were used sliver nanoparticles (AgNPs) at concentrations of 5and10mg L^{-1} and selenium nanoparticles (SeNPs) at concentrations of 2.5 and 5 mg L^{-1}. All media were supplemented with 30 g L^{-1} mannitol and 6g agar L^{-1} and autoclaved at 121°C for 15 min. Four explants were cultured on 50 ml of semi-solid medium and maintained in the growth chamber at 23-25°C in 16/8 h (light/dark),with 40-60µmol $m^{-2}s^{-1}$ provided by cool-white fluorescent lamps.After three weeks the sprouted buds were transferred to fresh media of the same composition and the sub-culture was performed every four weeks. Scores were given for necrosis and development as follows: Negative results = 1; below average = 2; average = 3, above average = 4 and excellent = 5 according to Pottino (1981). Treatments were arranged in a Completely randomized design. Each treatment was replicated three times, according to Snedecor and Cochron (1980). The obtained data were statistically analyzed and the means were difference according to Duncan multiple range test 1% level (Duncan, 1955).

Extraction of genomic DNA

0.5 g of fresh young olive leaves from the studied three cultivars ('Koroneiki', 'Picual' and 'Manzanillo') collected from silver (Ag) and selenium (Se) nanoparticles treated and untreated olive plants and were soaked in liquid nitrogen for DNA extraction using the 2% Cetyltrimethyl ammonium bromide (CTAB) procedure as described by Murry and Thompson, (1980) with modification by De la Rosa *et al*. (2002).

RAPD and DAMD profiles

Random amplified polymorphic DNA (RAPD) and Direct amplification of mini satellite-region DNA (DAMD) techniques of NPs treated and untreated olive plants were performed, using six RAPD and four DAMD primers as shown in Table (3) **(**Life Technologies, Gaithersburg, Md.). Each 25-µl amplification reaction containing 1X PCR reaction buffer, 2.0 mM $MgCl_2$, 0.2 mMdNTPs mix, 0.4 µM of each primer set, 1.5 U *Taq* DNA polymerase and adjusted to 25 µl using ddH_2O. Amplifications were carried out in DNA a thermo-cycler (Biometra, Germany) programmed as follows: 1 initial

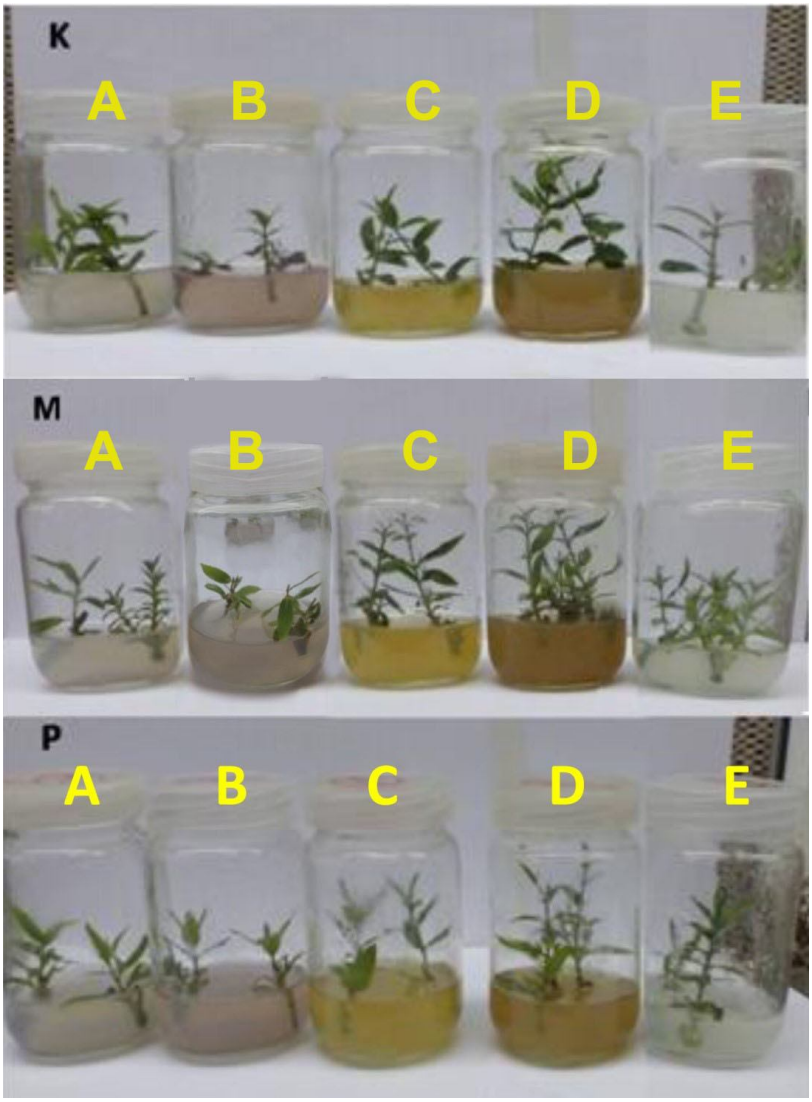

Fig. 1 : The effect of nanoparticles on different olive cultivars. K='Koroneiki', M= 'Manzanillo' and P = 'Picual'. A: 2.5 mg L^{-1} SeNPs; B:5 mg L^{-1}SeNPs; C:5 mg L^{-1}AgNPs; D:10 mg L^{-1}AgNPs and E: Control.

denaturation step at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 36°C for 1 min and 72°C for 1 min, and a final extension cycle at 72°C for 10 min.

Amplification product analysis

Amplification products were separated on a 1.5% agarose gel containing 1X TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) at 90 V. The genomic DNA was stained with RedSafe Nucleic Acid Staining Solution (1/20,000) (iNtRON Biotechnology, Inc. Kr). The gels were analyzed by UVI Geltec version 12.4, 1999-2005 (USA).

Data analysis

A matrix for RAPD and DAMD combined was generated by scoring reproducible bands as 1 for their presence and as 0 for their absence across the cultivar. Genetic similarity coefficients were computed according to Nei and Li (1979). The data were subsequently used to construct a dendrogram using the un-weighted pair group method of arithmetic averages (UPGMA) (Sneath and Sokal, 1973) employing sequential, agglomerative hierarchic and non-overlapping clustering (SAHN). All the computations were carried out using the software NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 2.02 (Rohlf, 2000). Jaccard's coefficients were calculated using similarity coefficients obtained from combined RAPD and DAMA profiles.

Results and Discussion

Effect of nanoparticles on different olive cultivars

Data in tables 1 and 2 shows the effect of using nanoparticle type and concentration on shoots of different olive cultivars at the proliferation stage. It was obtained

Table 1 : The effect of nanoparticle type and concentration on necrosis of different olive cultivars.

Treatment	Cultivar			
	Manzanillo	Picual	Koroneiki	Mean
Control	4.00	4.00	4.00	**4.00**
5 mg L^{-1}Silver	2.89	1.50	2.67	**2.35**
10 mg L^{-1}Silver	2.44	1.39	2.22	**2.02**
2.5 mg L^{-1}Selenium	1.89	1.20	1.89	**1.66**
5mg L^{-1}Selenium	1.49	1.00	1.56	**1.35**
Mean	**2.54**	**2.46**	**1.82**	
LSD of treatment	0.167			
LSD of cultivars	0.129			
LSD interaction	0.289			

Table 2 : The effect of nanoparticle type and concentration on development of different olive cultivars.

Treatment	Cultivar			
	Manzanillo	Picual	Koroneiki	Mean
Control	56.00	56.00	56.00	**56.00**
5 mg L^{-1}Silver	60.67	53.00	70.33	**61.33**
10 mg L^{-1}Silver	70.00	61.00	82.00	**71.00**
2.5 mg L^{-1}Selenium	94.00	65.33	97.00	**85.44**
5mg L^{-1}Selenium	82.67	70.00	94.33	**82.33**
Mean	**72.67**	**61.07**	**79.93**	
LSDof treatment	1.803			
LSD of cultivars	1.397			
LSD interaction	3.120			

that the addition of nanoparticle to the culture medium had a positive effect of olive explant compared with the control. They significantly reduced necrosis in the three olive cultivars and improved growth vigor and development of the cultured olive explants. AgNPs at 5 mg L^{-1} recorded the highest value of explant development, followed by AgNPs at 10 mg L^{-1}, while the control recorded the lowest value (fig. 1). Regarding the effect of nanoparticle on necrosis, AgNPs at both concentrations recorded the lowest value of explant necrosis, followed by SeNPs, while the control recorded the highest value. Several reports have proven positive effects of NPs on the shoot multiplication and the plant's growth *in vitro* (Aghdaei *et al*., 2012; Kumar *et al*., 2009). Silver nitrate ($AgNO_{3)}$ nanoparticles treatment increased survival and delayed explants senescence (Sarmast *et al*., 2015). $AgNO_3$ has shown to have important effects in plant tissue culture, improving micropropagation (Kotsias and Roussos, 2001; Naik and Chand, 2003) in many species. The inhibition of ethylene action or its production using $AgNO_3$ has shown stimulatory effects on plant regeneration (Palmer, 1992). The silver ion, a potent inhibitor of ethylene action (Songstad *et al*., 1988) has been reported to stimulate shoot regeneration in wheat and tobacco (Beyer, 1976) or in maize tissue culture (Purnhauser *et al*., 1987). Vannini *et al*. (2014) observed a toxic effect of 10 mg L^{-1} AgNPs on wheat seedlings at the early growth.

RAPD assay

Six decamer RAPD primers were used to study genotoxicity effect of AgNPs and SeNPs on three olive cultivars regenerated *in vitro* compared with untreated ones (fig. 2 and table 3). A total of 73 scorable bands, ranging from 100 (RAPD-4) to 2000bp (RAPD-2) were indicated using the six RAPD primers. The number of amplified productsper primer varied from nine (RAPD-5 and RAPD-6) to 21 (RAPD-2). Forty-three bands out of the 73 fragments were monomorphic (58.90%) and 30 reproducible bands were polymorphic (41.10%). The RAPD-5 recorded the highest polymorphism with 66.67% (table 3). In contrast, RAPD-3 and RAPD-6exhibited the lowest polymorphism (33.33%). Three out of the 73 were bands-specific (4.11%) (table 3). The olive plantlets cv 'Koroneiki' treated with 2.5 mg/L SeNPs scored one positive marker of +700 bp, using primer RAPD-4. Moreover, 'Koroneiki' and 'Picual' cultivars treated with 5 mg/L SeNPs revealed one allele of +590 bp, using primer RAPD-2. Furthermore, the control plantlets cv 'Koroneiki' recorded one amplicon of +300 bp, using primer RAPD-2 and absent in the other controls and treatments (table 3).

DAMD profiles

Four DAMD primers amplified genomic DNA extracted of NPs treated and untreated olive plantlets. A number total of 41 alleles were recorded using four primers DAMD (fig. 3 and table 4). The number of amplicons varied from 100bp (primer DAMD-3) to 1500bp (primer DAMD-4). Twenty-four out of 41 were monomorphic (58.54%), 17 amplified fragments were polymorphic (41.46%). The maximum number of loci was showed in primers DAMD-2, DAMD-3 and DAMD-4 (11 loci), while the minimum number of loci was observed in primer DAMD-1 (eight bands).The DAMD-4scored

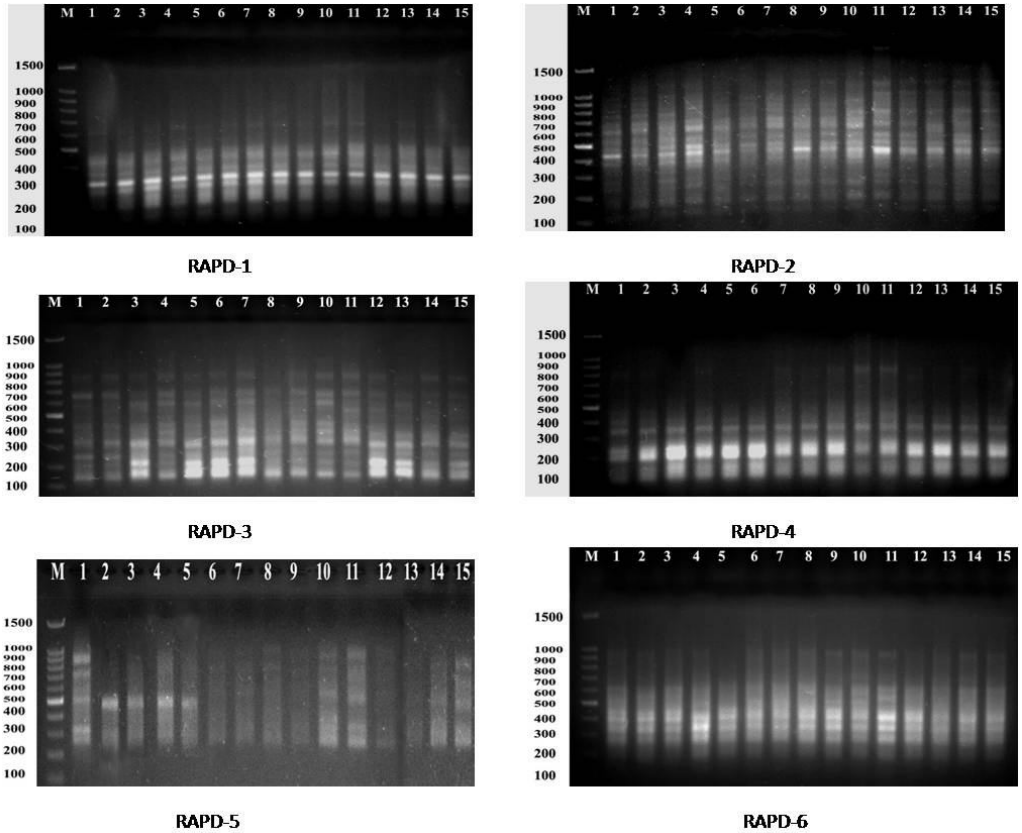

Fig. 2: RAPD-PCR analysis of three olives cultivars treated with selenium and silver nanoparticles. Lane M = 100 bp DNA ladder. Lane 1= (‘Picual’, control); lane 2= (‘Picual’, 2.5 mg/L SeNPs); lane 3= (‘Picual’, 5 mg/L SeNPs), lane 4= (‘Picual’, 5 mg/L AgNPs); lane 5= (‘Picual’, 10 mg/L AgNPs); lane 6= (‘Manzanillo’, control), lane 7= (‘Manzanillo’ ,2.5 mg/L SeNPs); lane 8= (‘Manzanillo’, 5 mg/L SeNPs); lane 9= (‘Manzanillo’, 5 mg/L AgNPs); lane 10= (‘Manzanillo’, 10 mg/L AgNPs); lane 11=(‘Koroneiki’, control); lane 12= (‘Koroneiki’, 2.5 mg/L SeNPs); lane 13= (‘Koroneiki’, 5 mg/L SeNPs), lane 14= (‘Koroneiki’, 5 mg/L AgNPs) and lane 15= (‘Koroneiki’, 10 mg/L AgNPs).

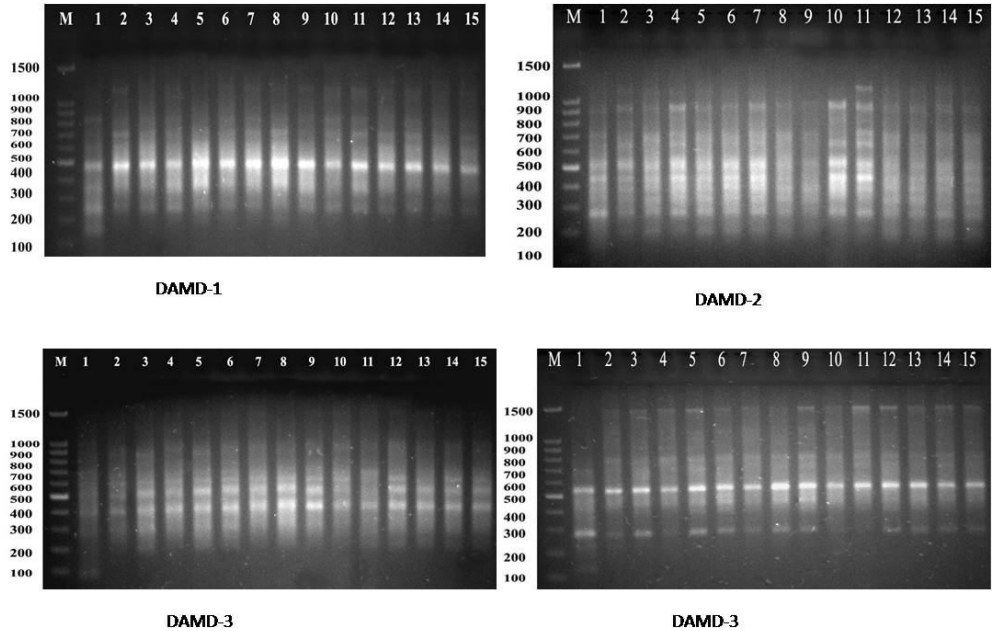

Fig. 3 : DAMD profiles of three olives cultivars treated with selenium and silver nanoparticles. Lane M= 100 bp DNA ladder. Lane 1= (‘Picual’, control); lane 2= (‘Picual’, 2.5 mg/L SeNPs); lane 3= (‘Picual’, 5 mg/L SeNPs), lane 4= (‘Picual’, 5 mg/L AgNPs); lane 5=(‘Picual’, 10 mg/L AgNPs); lane 6= (‘Manzanillo’, control), lane 7= (‘Manzanillo’, 2.5 mg/L SeNPs); lane 8= (‘Manzanillo’, 5 mg/L SeNPs); lane 9= (‘Manzanillo’, 5 mg/L AgNPs); lane 10= (‘Manzanillo’, 10 mg/L AgNPs); lane 11= (‘Koroneiki’, control); lane 12= (‘Koroneiki’, 2.5 mg/L SeNPs); lane 13= (‘Koroneiki’, 5 mg/L SeNPs), lane 14= (‘Koroneiki’, 5 mg/L AgNPs) and lane 15= (‘Koroneiki’, 10 mg/L AgNPs).

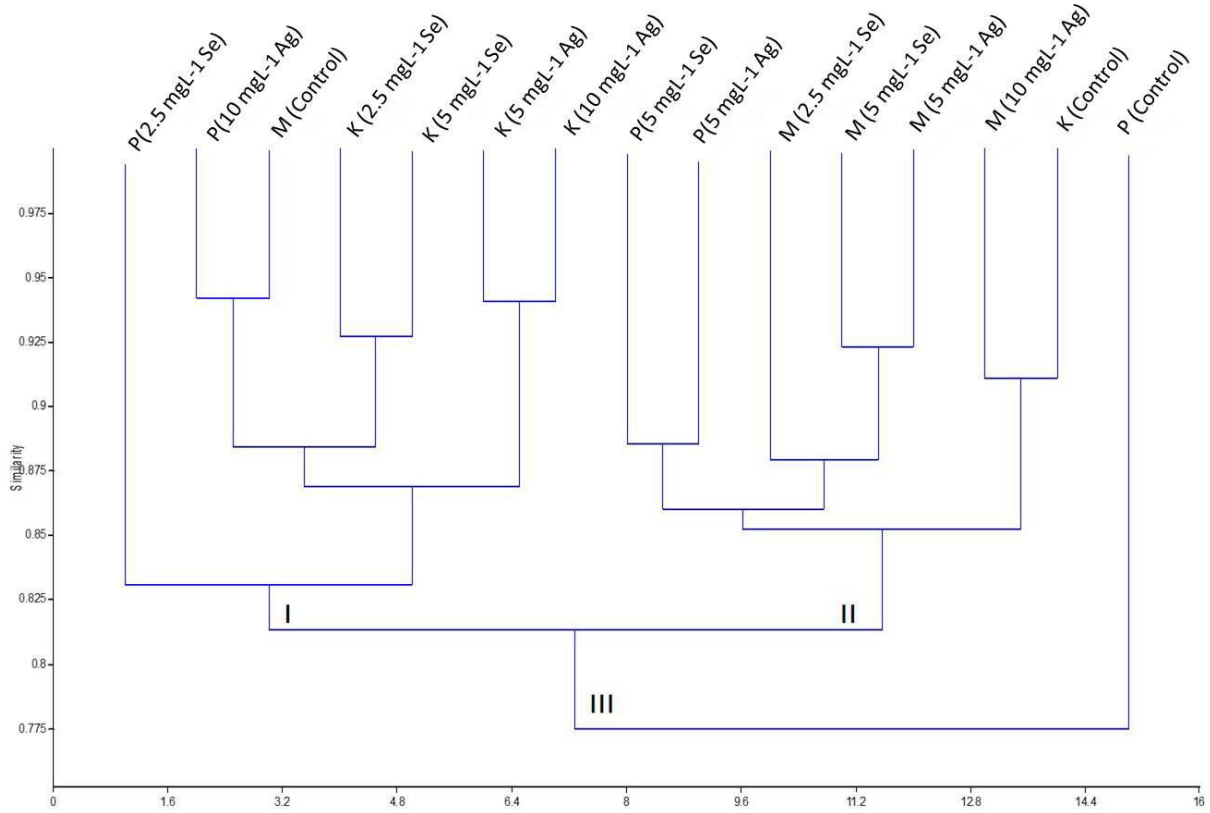

Fig. 4 : Dendrogram of three olives cultivars treated with selenium and silver nanoparticles depend on combined RAPD and DAMD by UPGMA cluster analysis from the similarity matrix obtained. K= 'Koroneiki', M= 'Manzanillo' and P= 'Picual'.

Table 3 : RAPD-PCR analysis of three olives cultivars treated and untreated with silver and selenium nanoparticles.

Primer Code No.	Primer sequences	Size range of the scorableloci (bp)	Total loci	No. of monomorphic loci	No. of polymorphic loci	% polymorphism	Unique loci	Molecular size of markers (bp)
RAPD-1	CAGGCCCTTC	130-1200	11	6	5	45.45	0	-
RAPD-2	TGCCGAGCTG	120-2000	21	13	8	38.10	2	+300; +590
RAPD-3	CCCGTCAGCA	156-1310	12	8	4	33.33	0	-
RAPD-4	GGGTAACGCC	100-820	11	7	4	36.36	1	+700
RAPD-5	GTGTCGCGAG	285-920	9	3	6	66.67	0	-
RAPD-6	CAGCACCCAC	280-1420	9	6	3	33.33	0	-
Total	**-**	**100-2000**	**73**	**43(58.90%)**	**30(41.10%)**	**41.10%**	**3**	**4.11%**

Table 4 : DAMD analysis of three olives cultivars untreated and treated with silver and selenium nanoparticles.

Primer Code no.	Primer sequences	Size range of the scorable loci (bp)	Total loci	No. of monom-orphic loci	No. of polymor-phic loci	% polymor-phism	Unique loci	Molecular size of markers (bp)
DAMD-1	GGTGTAGAGAGGGGT	155-1330	8	5	3	37.50	2	-1250; +155
DAMD-2	CCTCCTCCCTCCT	169-1270	11	9	2	18.18	0	-
DAMD-3	GGAGGTTTTCA	100-1450	11	6	5	45.45	2	+100; +1000
DAMD-4	GAGGGTGGCGGTTCT	129-1500	11	4	7	63.64	3	+129; -810;,+1451
Total	**-**	**100-1500**	**41**	**24(58.54%)**	**17**	**41.46%**	**7**	**17.07%**

Table 5. Similarity index among the three olives cultivars treated with silver and selenium nanoparticles by combined RAPD and DAMD.

Cultivar / Treatment	Picual (Control)	Picual (2.5 mg/L SeNPs)	Picual (5 mg/L SeNPs)	Picual (5 mg/L Ag NPs)	Picua (10 mg/L Ag NPs)	Manza-nillo (control)	Manza-nillo (2.5 mg/L SeNPs)	Manza-nillo (5 mg/L SeNPs)	Manza nillo (5 mg/L AgNPs)	Manza nillo (10 mg/L AgNPs)	Koroneiki (control)	Koroneiki (2.5 mg/L SeNPs)	Koroneiki (5 mg/L SeNPs)	Koroneiki (5 mg/L Ag NPs)	Koroneiki (10 mg/L AgNPs)
P (control)	1.00														
Picual (2.5 mg/L SeNPs)	0.81	1.00													
Picual (5 mg/L SeNPs)	0.75	0.88	1.00												
Picual (5 mg/L Ag NPs)	0.79	0.83	0.89	1.00											
Picual (10 mg/L Ag NPs)	0.80	0.84	0.84	0.83	1.00										
Manzanillo (control)	0.76	0.84	0.85	0.80	0.94	1.00									
Manzanillo (2.5 mg/L SeNPs)	0.73	0.84	0.88	0.87	0.84	0.87	1.00								
Manzanillo (5 mg/L SeNPs)	0.79	0.81	0.85	0.85	0.89	0.88	0.90	1.00							
Manzanillo (5 mg/L Ag NPs)	0.82	0.84	0.84	0.88	0.88	0.85	0.85	0.92	1.00						
Manzanillo (10 mg/L Ag NPs)	0.76	0.80	0.83	0.90	0.76	0.75	0.85	0.84	0.87	1.00					
Koroneiki (control)	0.74	0.79	0.83	0.87	0.76	0.77	0.88	0.82	0.84	0.91	1.00				
Koroneiki (2.5 mg/L SeNPs)	0.74	0.86	0.86	0.81	0.91	0.90	0.82	0.83	0.82	0.74	0.74	1.00			
Koroneiki (5 mg/L SeNPs)	0.74	0.82	0.84	0.79	0.88	0.85	0.80	0.82	0.79	0.74	0.72	0.93	1.00		
Koroneiki (5 mg/L Ag NPs)	0.79	0.81	0.79	0.84	0.87	0.84	0.79	0.86	0.85	0.80	0.76	0.89	0.89	1.00	
Koroneiki (10 mg/L Ag NPs)	0.80	0.80	0.78	0.83	0.86	0.84	0.78	0.85	0.84	0.80	0.79	0.86	0.88	0.94	1.00

NPs= Nanoparticles.

the highest polymorphism with 63.64%, followed by primer DAMD-3 (45.45%).On the contrary; primer DAMD-2 exhibited the lowest polymorphism (18.18%).Seven out of the 41 were markers (17.07%) (table 4). The control plantlets cv 'Picual' appeared two negative markers of -1250 and -810 bp, using DAMD-1 and DAMD-4, respectively which revealed in all other treatments. In addition, 'Picual' control induced four positive markers with molecular sizes (+100 and +1000bp), (+129 bp), (+155bp), using primers DAMD-3, DAMD-4 and DAMD-1, respectively. Besides, 'Picual' plantlets treated with 2.5 mg/L SeNPs showed one specific band with molecular size+1451 bp, using primer DAMD-4 (table 4).

In the current study, RAPD and DAMD profiles of screening DNA change, induced by non-lethal levels of olive plantlets subject to different concentrations of Ag and Se NPs. Differencesin RAPD and DAMD assays were analyzed depending on the appearance or disappearance of bands. Amplification profiles of genomic DNA with molecular markers indicating that olive plantlets treated with 5 and 10 mg L^{-1}AgNPs and 2.5 and 5 mg L^{-1} SeNPs scored polymorphism, which ranged from 41.10 to 41.46% using RAPD and DAMD assays, respectively. Novel fragments were obtained when genomic DNA amplified from the olive plants treated with nanoparticles based on the nanomaterial concentration and primer. These results were in agreement with Singh *et al*. (2009) found that RAPD-PCR profiles supply a simple and fast way to study genotoxicity on onion plants, compared with conventional ways, this can bean analysis of a big number of plants through short time. It can be concluded from the study that 10 mgL^{-1} NiO-NP changes the DNA in the RAPD-PCR profiles, compared to untreated plants. In addition, any alteration in the banding patterns by the appearance or disappearance of bands must be is taken to be a measure of polymorphism (Liu *et al*., 2007). It supposed that NiONPs causes DNA damage by modification and oxidization of nucleotide bases, DNA double-strand breaks, changed DNA-protein cross-linking, point mutation, bulky adducts, altered gene expression and an introduction of basic sites, all of which affect DNA amplification and primer binding in RAPD-PCR analysis. The NPs are shown to produce genotoxicity involve metal/ metal-oxide NPs such asFeO_4, AgO, CuO and

TiO_2, carbon nanotubes and fullerenes, metal oxide NPs which inhibited growth of the plants(Rodriguez*et al*., 2011; Srivastava *et al*., 2015). Several studies have demonstrated the phytotoxicity of NPs applied at higher concentrations to plant tissue (Craig and Jason, 2011; Ghodake *et al*., 2011). Ewais *et al*. (2015) reported that the addition of AgNPs to tissue culture medium stimulates changes in the callus morphology and anatomy and alter DNA and protein profiles. Moreover, López-Moreno *et al*. (2010) observed damage in the genomic DNA structure in soybean plants due to treatment with CeO_2NPs by RAPD-PCR assay.

Cluster analysis

The genetic distances (GDs) among three olive cultivars treated with NPs and the control ranged from 0.72 to 0.94 based on combined RAPD and DAMD profiles. The cluster analysis using UPGMA based on genetic distances of markers found that the three olive cultivars could be divided into three major clusters (fig. 4). These results were in agreement with Hassan *et al*. (2017) found that the genetic distances (GDs) among three olive cultivars regenerated *in vitro* and the donor plants ranged from 0.74 and 0.94 depending on RAPD-PCR analysis.

The first cluster (I): (similarity range of 0.72 to 0.94) composed of 'Picual' (P) treated with 2.5 mg/L SeNPs and 10mg/L AgNPs, 'Koroneiki' (K) (2.5 and 5mg/L SeNPs and 5 and 10 mg/L AgNPs) and 'Manzanillo' (M) (the control). The second cluster (II): (similarity range of 0.73 to 0.92) involved into 'Picual' (P) treated with 5 mg/L SeNPs and 5 mg/LAgNPs, 'Manzanillo' (M) treated at (2.5 and 5 mg/L SeNPs and 5 and 10 mg/L AgNPs) and 'Koroneiki'(K) control. The third cluster (III): (similarity range of 0.73 to 0.82 similarity) consisted of 'Picual' (P) control (table 5 and fig. 4). The cluster analysis is one of the important ways in numerical analysis concerning fragment recording and analysis of RAPD fingerprinting. In this study, cluster analysis was done to evaluate the level of DNA polymorphism between the NPs treated and untreated olive plants. A dendrogram was constructed using the distance matrix by using UPGMA method observed that olive plantlets treated with high concentrations of selenium and silver NPs induced changes on DNA level depending on the NPs concentration.

Conclusion

This study is the first report stated that5mg L^{-1}AgNPs had a positive effect on *in vitro* performance. However, 10 mg L^{-1} AgNPs and 2.5 and 5 mg L^{-1} SeNPs induced changes on genomic DNA level. In addition, RAPD and DAMD assays can be successfully applied as a sensitive method of detecting DNA stability and genotoxicity in olive plants treated with different concentrations of selenium and silver NPs.

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